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Abstract

There has been fantastic progress in solving GPCR crystal structures. However, the ability of X-ray crystallography to guide the drug discovery process for GPCR targets is limited by the availability of accurate tools to explore receptor-ligand interactions. Visual inspection and molecular mechanics approaches cannot explain the full complexity of molecular interactions. Quantum Mechanics approaches (QM) are often too computationally expensive, but the Fragment Molecular Orbital (FMO) method offers an excellent solution that combines accuracy, speed and the ability to reveal key interactions that would otherwise be hard to detect. Integration of GPCR crystallography or homology modelling with FMO reveals atomistic details of the individual contributions of each residue and water molecule towards ligand binding, including an analysis of their chemical nature.

Keywords	GPCR; FMO; QM;G-protein coupled receptors; Structure-based drug-discovery; Interactions; Drug-discovery
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GPCR–Ligand Interactions Using a Fragment Molecular Orbital-Based Approach

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Abstract

There has been fantastic progress in solving GPCR crystal structures. However, the ability of X-ray crystallography to guide the drug discovery process for GPCR targets is limited by the availability of accurate tools to explore receptor-ligand interactions. Visual inspection and molecular mechanics approaches cannot explain the full complexity of molecular interactions. Quantum Mechanics approaches (QM) are often too computationally expensive, but the Fragment Molecular Orbital (FMO) method offers an excellent solution that combines accuracy, speed and the ability to reveal key interactions that would otherwise be hard to detect. Integration of GPCR crystallography or homology modelling with FMO reveals atomistic details of the individual contributions of each residue and water molecule towards ligand binding, including an analysis of their chemical nature.

Introduction

G-protein coupled receptor (GPCR) - ligand interactions are fundamental to almost all processes occurring in living organisms and as such it is perhaps unsurprising that they are the targets of about 40% of all prescribed drugs [1]. What is surprising is that these drugs only target around 50 of the 800 known GPCRs [2]. Thus, there is huge potential in terms of the number of new therapeutic targets within this family [1,2]. Further progress in GPCR drug discovery is highly dependent on the availability of the protein structural information and an understanding of the interactions between the receptors and small molecule drug candidates [3].

Recent breakthroughs in structural biology have resulted in the solution of over 260 structures of GPCR-ligand complexes comprising more than 50 unique GPCRs [4] (http://gpcrdb.org/structure/statistics [5] or https://zhanglab.ccmb.med.umich.edu/GPCR-EXP/. However, the analysis of molecular interactions in the atomic-resolution structures is usually performed either by 'visual inspection' or with simple molecular mechanics (MM) models that cannot accurately explain the full complexity of those interactions [6] or their chemical nature. In many cases, the mechanisms by which a particular ligand interacts with its receptor remain unclear, making rationalization of affinity measurements challenging.

Recently, several notable reports have been published [6-9] that emphasized the crucial role of a large number of 'non-obvious' interactions in receptor-ligand binding. These include CH/π [10,11], halogen/π [12], cation/π [13] and non-classical hydrogen bonds [14], which are typically not properly parameterized in currently available force fields (FF) [8]. Furthermore, the role of hydrophobic interactions is vital for biomolecular recognition but there is still no reliable non-QM predictive method for its quantification [6]. Historically, hydrophobic interactions have been accounted for with terms that are delocalized, typically in the form of either a shape complementarity term or solvation/entropy penalty. Such approaches are useful

for providing an overall estimation of the contribution to the affinity, but do not readily provide spatial decomposition that can be integrated into a design strategy for the development of future compounds. Visual inspection of the interactions formed between two neighboring aromatic moieties taking into account their geometries [15,16] and substitutions [17] is usually quite subjective. The strength of these interactions can be evaluated [18] with quantum mechanical methods, which are considered to be a reliable approach for the exploration of receptor-ligand interactions [19,20] and an improvement over dispersion potentials in FF. However, despite their many advantages, traditional QM methods are generally not feasible for large biological systems due to their high computational cost [21]. This drawback can be addressed using low scaling QM approaches like the fragment molecular orbital method [11,20,22,23] as well as others [21,24]

The fragment molecular orbital approach

FMO offers a considerable computational speed-up over traditional QM methods [25]. One of the key features of the FMO approach is the provision of a list of the interactions formed between a receptor and a ligand. Quantification of the strength of these interactions and a characterisation of their chemical nature is provided [21]. Such information is essential for medicinal chemists to be able to execute a rational approach to compound modification in order to increase favourable interactions. Furthermore, an understanding of the regions of the binding site that have the most prominent contributions is likely to be useful in understanding the origins of functional efficacy.

FMO involves partitioning the system into smaller pieces called fragments (Figure 1). For example, in receptors, each residue can be represented as a fragment. Similarly, the ligand can be represented by a single or multiple fragments as necessary. The FMO pair interaction energy (PIE) between any two fragments is a sum of four terms: electrostatics, exchange-repulsion, charge transfer and dispersion. It is calculated using pair interaction energy decomposition analysis (PIEDA) [26]. The electrostatic and charge transfer terms are

important in salt-bridges, hydrogen bonds and polar interactions, whilst the dispersion term can be considered to be more hydrophobic in nature. The exchange-repulsion term describes the steric repulsion between electrons [21] that prevents atoms from collapsing into each other.

By performing QM calculations on fragments, one can achieve a high level of accuracy with very high efficiency. Several QM packages, including GAMESS [27], ABINIT-MP [28] and PAICS [29], contain modules for performing FMO calculations.

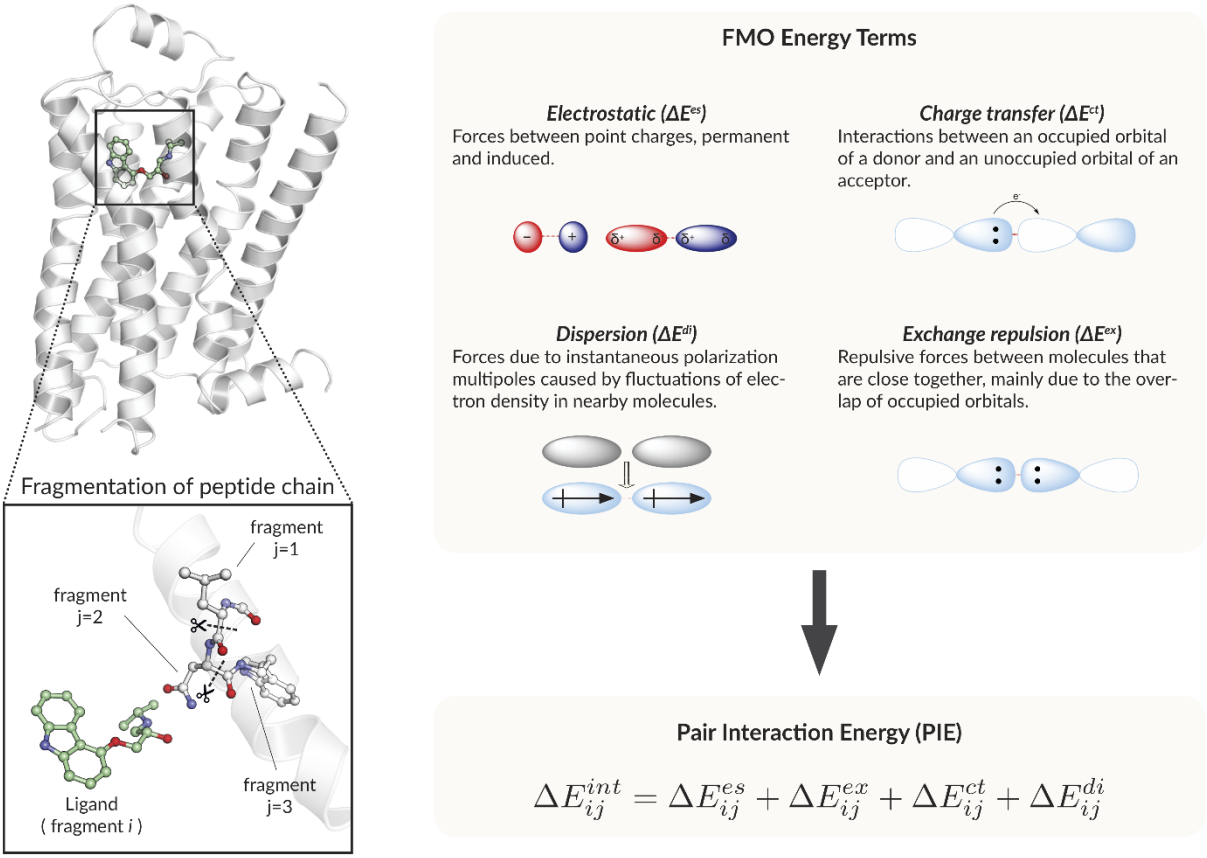

Figure 1: Workflow for PIEDA calculations and details on each of the PIE terms that are computed [21]. The electrostatic component arises from the Coulomb interaction between polarised charge distributions of fragments. The exchange-repulsion term is derived from the interaction between fragments situated in close proximity and is always repulsive; it is due to the Pauli repulsion and is related to the overlap of two occupied orbitals. The charge transfer term arises from the interaction between occupied orbitals of a donor and unoccupied orbitals of an acceptor. The dispersion term arises from the interaction between instantaneous dipole moments of two fragments. It is obtained in PIEDA from the correlation energy of electrons.

The key difference between FMO and MM methods is the fact that FMO takes into account polarization and charge transfer [11,30]. The description of electrostatics in most force fields is based on static charges that neglect polarization and in systems such as proteins this is an approximation to the actual state. Van-der-Waals forces, despite being generally well parameterized, are not capable of detecting the directional nature of the dispersion terms involving halogens [31]. Reported examples [32] comparing FMO and MM, have shown that the FMO method clearly outperformed FF-based scoring functions and demonstrated a high correlation with experimentally measured values of protein-ligand affinity [32,33]. It is no longer necessary to compromise in performing detailed analysis of protein-ligand structures using MM/FF when a similar analysis can be done with FMO that is reasonably quick [34]. A typical FMO calculation on a suitably truncated ligand-receptor complex takes approximately 4h on a cluster with 36 CPU cores. This time can be reduced to a matter of seconds when FMO is combined with the DFTB method [34].

According to our previous report [32] FMO demonstrated that it can produce accurate results even for crystal structures with low (> 3.0Å) resolution. This suggests that FMO can even be applied to homology models [35,36].

Exploring GPCR ligand interactions with FMO

In our recent report [32] we described how FMO has been applied to the analysis of 18 GPCR-ligand crystal structures representing different branches of the GPCR genome. This work revealed key and consensus interactions that are involved in receptor-ligand binding and that were previously omitted from structure-based descriptions. These included hydrophobic interactions, non-classical hydrogen bonds and the involvement of backbone atoms. It was revealed that in many cases electrostatic and hydrophobic contributions to the receptor-ligand binding energy have equal magnitudes. We were also able to demonstrate general trends in ligand binding. For example, residues in positions 3.32, 3.33, 6.48, 6.51, 6.52, 7.39 and 7.43

(according to the Ballesteros and Weinstein numbering scheme [37,38]), located on helices 3, 6 and 7, frequently make considerable contributions to receptor-ligand binding [32]. The residues in positions 3.33, 6.48, 6.51, 6.52 and 7.43 form interactions with mainly hydrophobic character while the residues in positions 3.32 and 7.39 form mainly electrostatic interactions. These observations were in agreement with site-directed mutagenesis data and previous reports [39] that residues in these positions frequently make contact with diverse ligands across nearly all class A GPCRs. Residues in other positions were less frequently involved, only forming interactions with specific ligands [39]. These key positions in helices 3, 6 and 7 form a consensus core of the ligand-binding pocket, even though the amino acids present therein are not conserved (average identity < 41%). Variation in the amino acids occupying the topologically equivalent positions contributes to ligand specificity across different GPCRs.

FMO and its application to GPCR structure-based drug design

FMO can be a highly useful tool for rational structure-based drug design (SBDD) [40,41] as it provides an accurate and comprehensive list of strong, weak and repulsive interactions between a ligand and its surrounding residues. Such information is highly instructive in rational SBDD for guiding modifications such as scaffold replacement/scaffold hopping, linking (specifically in the case of fragment-based drug discovery [33]), extension of chemical moieties to form stronger or new interactions with the protein or alterations to remove repulsions [40,42].

To illustrate the typical results obtained through FMO we performed calculations on the complex between the human β_2-adrenergic receptor (β_2AR) and the inverse agonist Carazolol (PDB entry 2RH1, Figure 2a), and between β_2AR and the agonist BI-167107 (PDB entry 4LDE, Figure 2b). We consider any interaction with an absolute PIE greater than or equal to 3.0 kcal/mol to be significant [43]. A comparison of the interactions formed by these two ligands with β_2AR is shown in Figure 2c. FMO indicated that the two molecules shared six

interactions, four of which were more strongly implicated in the agonist binding and two were stronger in the binding of the inverse agonist.

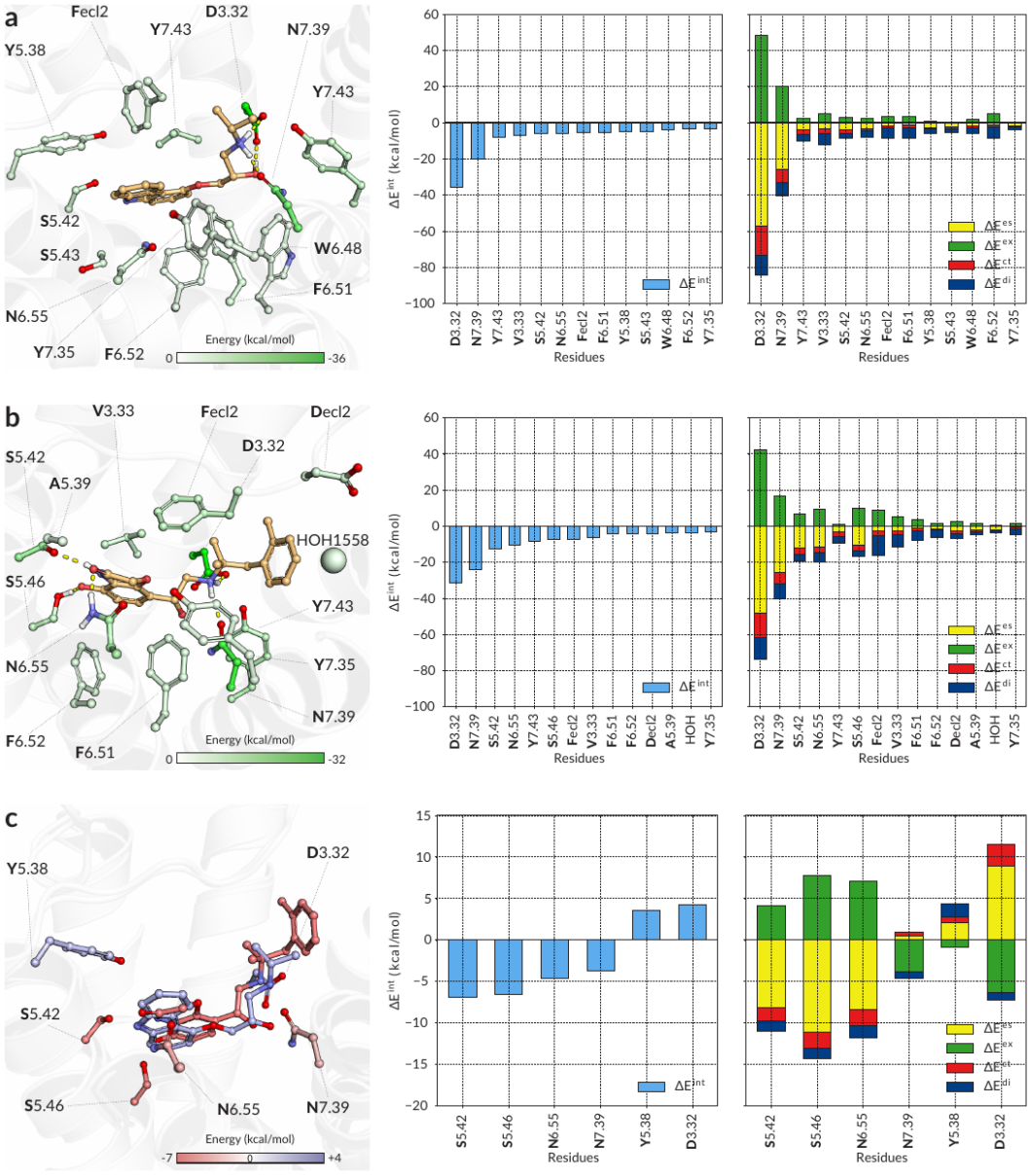

Figure 2. FMO calculations for two β_2AR-ligand complexes. Residues are numbered according to the Ballesteros Weinstein indexing scheme where ecl stands for extracellular loop. **(a)** Inverse agonist Carazolol (PDB entry 2RH1 [44]) **(b)** Agonist BI-167107 (PDB entry 4LDE [45]). The carbon atoms of the ligands are shown in light orange and the receptor residues are colored according to the PIE values calculated by FMO. Nitrogen atoms are shown in blue, oxygen in red, sulfur in yellow and chlorine in light green. The classical hydrogen bonds between the receptor and the ligand are marked as yellow dashed line. The

left-hand plots show the total PIE for the residues whilst those on the right show the PIEDA of these interactions. PIE terms: electrostatics, dispersion, charge-transfer and exchange-repulsion are color coded yellow, blue, red and green, respectively. **(c)** Shared interactions between the Carazolol and BI-167107-β_2AR complexes. In this case, Carazolol is show in light blue and BI-167107 in salmon pink, with the residues interacting more strongly with Carazolol shown on a white to light blue spectrum and the residues interacting more strongly with BI-167107 on a white to red spectrum. $\Delta E^{Int} = PIE^{\textbf{BI-167107}} - PIE^{\textbf{Carazolol}}$.

FMO can also be applied to the analysis of ligand-water-protein networks [46], to distinguish between energetically favourable and unfavourable water molecules. This enables the rational design of ligands to interact with or displace certain waters. As previously demonstrated [32], significant correlation between protein-ligand affinities and FMO energy terms [33] suggests that they can be efficiently used as descriptors in QSAR modelling to predict the binding affinities of new molecules.

In our experience, application of the FMO method in the hit-to-lead and lead optimization stages of drug discovery is a highly valuable approach for the design, evaluation and filtering of targets for synthesis or for analysis of structure–activity relationship (SAR) [40,47]. The FMO approach can be particularly useful for in depth analysis of crystal-structures and discerning the exact chemical nature of particular interactions between a receptor and a ligand.

There are ongoing efforts to improve the quality of FMO predictions in a number of ways, including (i) analysis of solute-solvent interactions using the solvation model density combined with the fragment molecular orbital method [48], (ii) empirical corrections and pair interaction energies in the fragment molecular orbital method [49] and (iii) pair interaction energy decomposition analysis for density functional theory and density functional tight-binding with an evaluation of energy fluctuations in molecular dynamics [50].

FMO drug-design consortium (FMODD) and FMO database (FMO-DB)

We anticipate that the FMO approach will be used to provide further insights into protein-ligand and protein-protein interactions, with a view to rationally designing novel therapeutic compounds. To achieve these goals and to make FMO even more applicable to structural analysis and drug discovery it is very beneficial to combine the research efforts of the academic and industrial sectors, as has been demonstrated by CompBioMed (https://www.compbiomed.eu/) and the FMO drug-design consortium (FMODD). FMODD (http://eniac.scitec.kobe-u.ac.jp/fmodd, the English site is currently under construction) is an industry-academia-government collaborative consortium originally established in Japan by Prof Kaori Fukuzawa (Hoshi University), Prof Shigenori Tanaka (Kobe University) and Dr Teruki Honma (RIKEN). FMODD is focused on the development and use of FMO-based computational methods for drug discovery, such as the auto-FMO protocol [51].

The FMODD collaborative endeavor has also resulted in the generation of an FMO database (FMO-DB). FMO-DB contains FMO results (including PIEDA) for >1,000 protein-ligand complexes extracted from the Protein Data Bank. FMO-DB (http://drugdesign.riken.jp/FMODB) is scheduled to be publicly available from March 2019 with a user-friendly interface for search and analysis. An example entry from FMO-DB is shown in Figure 3.

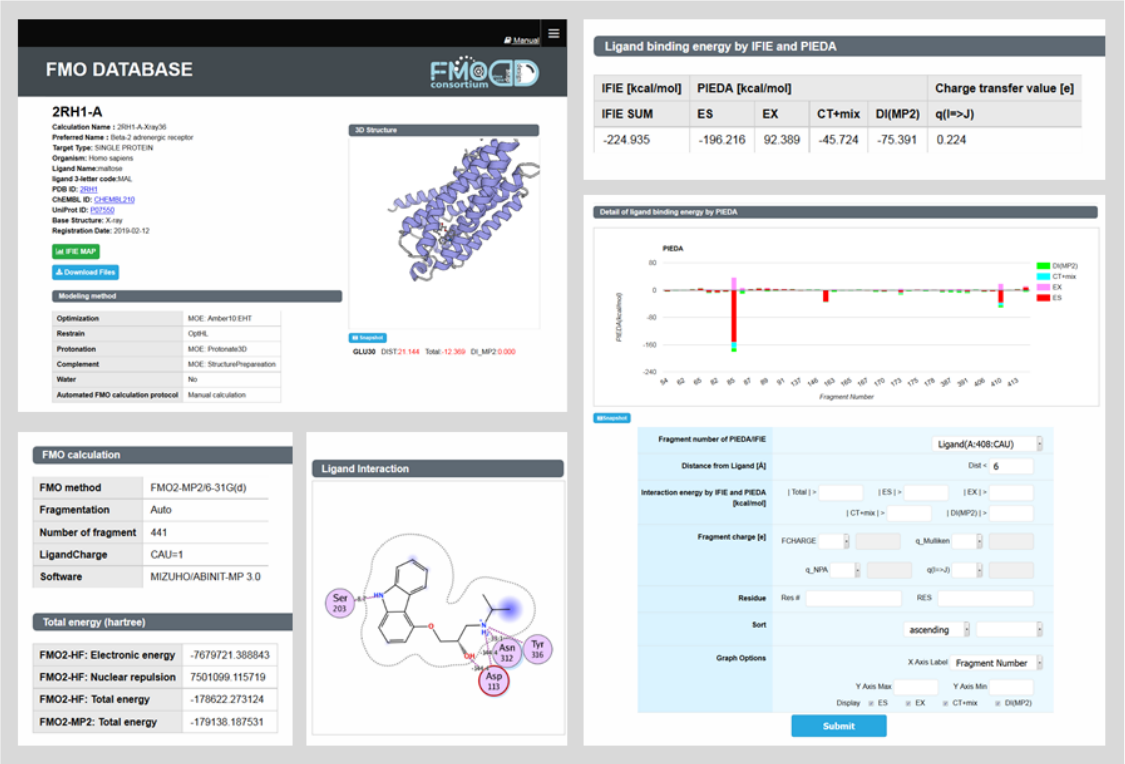

Figure 3. Example screenshot of the FMO-DB interface showing a calculation for the crystal structure of the human β_2-adrenergic receptor bound to Carazolol (PDB entry 2RH1)

The FMODD collaboration and its FMO-DB provide a good example of how collaboration between researchers can lead to the development of new FMO applications and generate a knowledge base for the benefit of everyone, preventing duplication of effort and making research more efficient. Application of the FMO-DB to the characterization of GPCR-ligand interactions will enable researchers to characterise the individual contributions that receptor residues and water molecules make towards ligand binding for their GPCR of interest, greatly improving the prospects of expanding the number of GPCRs used as therapeutic targets in the future.

Conclusions

The FMO approach has a proven record in the deep analysis of crystal-structures and in characterizing the interactions between receptors and ligands [32]. FMO brings the power

of general *ab initio* QM approaches to molecular biochemical research: the calculations are reasonably easy to set up and can be performed on moderate PC clusters within an acceptable time scale. FMO provides insights into the chemical nature of interactions that are normally difficult to detect with non QM methods. FMO analysis can result in two considerable benefits: (a) complex QM theories are condensed into four simple and intuitively clear quantities, and (b) calculations become much faster than traditional QM approaches. This information can be used to understand the chemical nature of existing receptor-ligand complexes, which in turn can be used to guide mutagenesis experiments or to help optimize ligands in ways that were previously not considered [43,47]. There are increasing effort to extend the application of FMO to structural optimization, protein-protein interactions, molecular dynamics simulations [52] and many others.

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Declaration of Interest

Declarations of interest: none

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- •• of outstanding interest

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•• In this important study, the electronic properties of bioactive proteins were analyzed using FMO methodology in solution: coupling with an implicit solvent model based on the Poisson-Boltzmann surface area called FMO-PBSA. The predicted ligand-binding affinities of bioactive compounds to protein target showed a good correlation with their experimentaly measured activities. Authors demostarted that FMO-PBSA approach is a promising tool both for exploartion of protein-ligand interactions and affinity prediction

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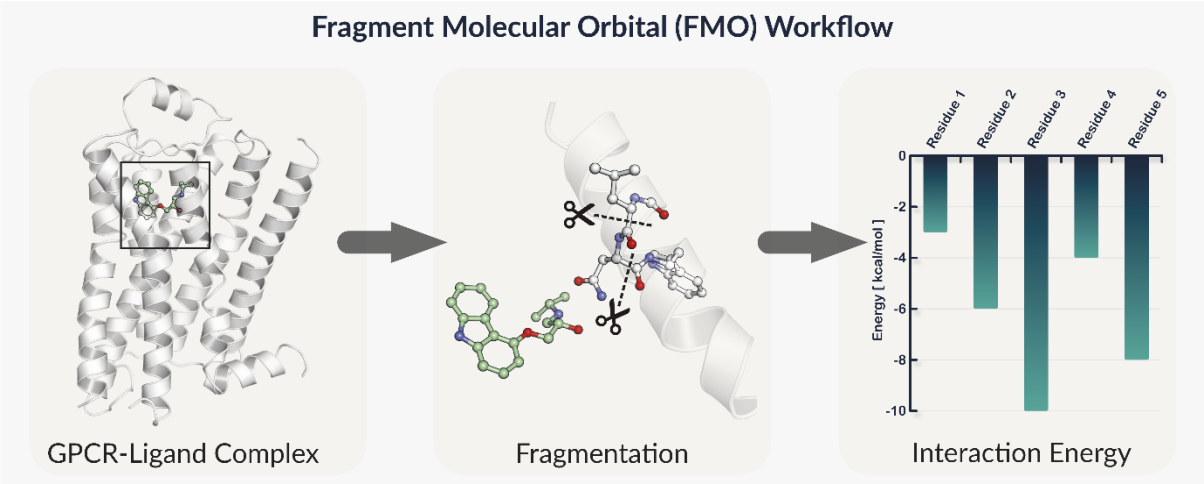
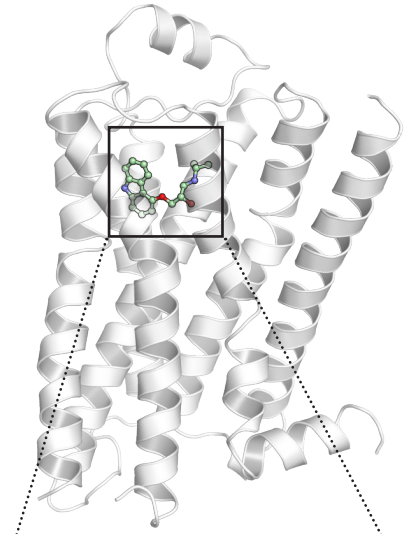
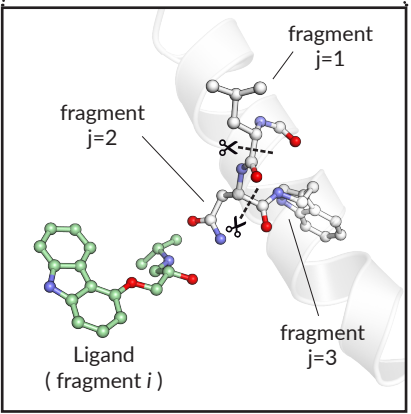


Graphical abstract



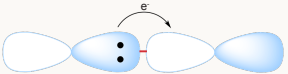
Fragmentation of peptide chain



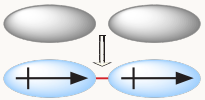
FMO Energy Terms

Electrostatic (ΔE^{es})
Forces between point charges, permanent and induced.

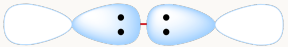
Charge transfer (ΔE^{ct})
Interactions between an occupied orbital of a donor and an unoccupied orbital of an acceptor.



Dispersion (ΔE^{di})
Forces due to instantaneous polarization multipoles caused by fluctuations of electron density in nearby molecules.

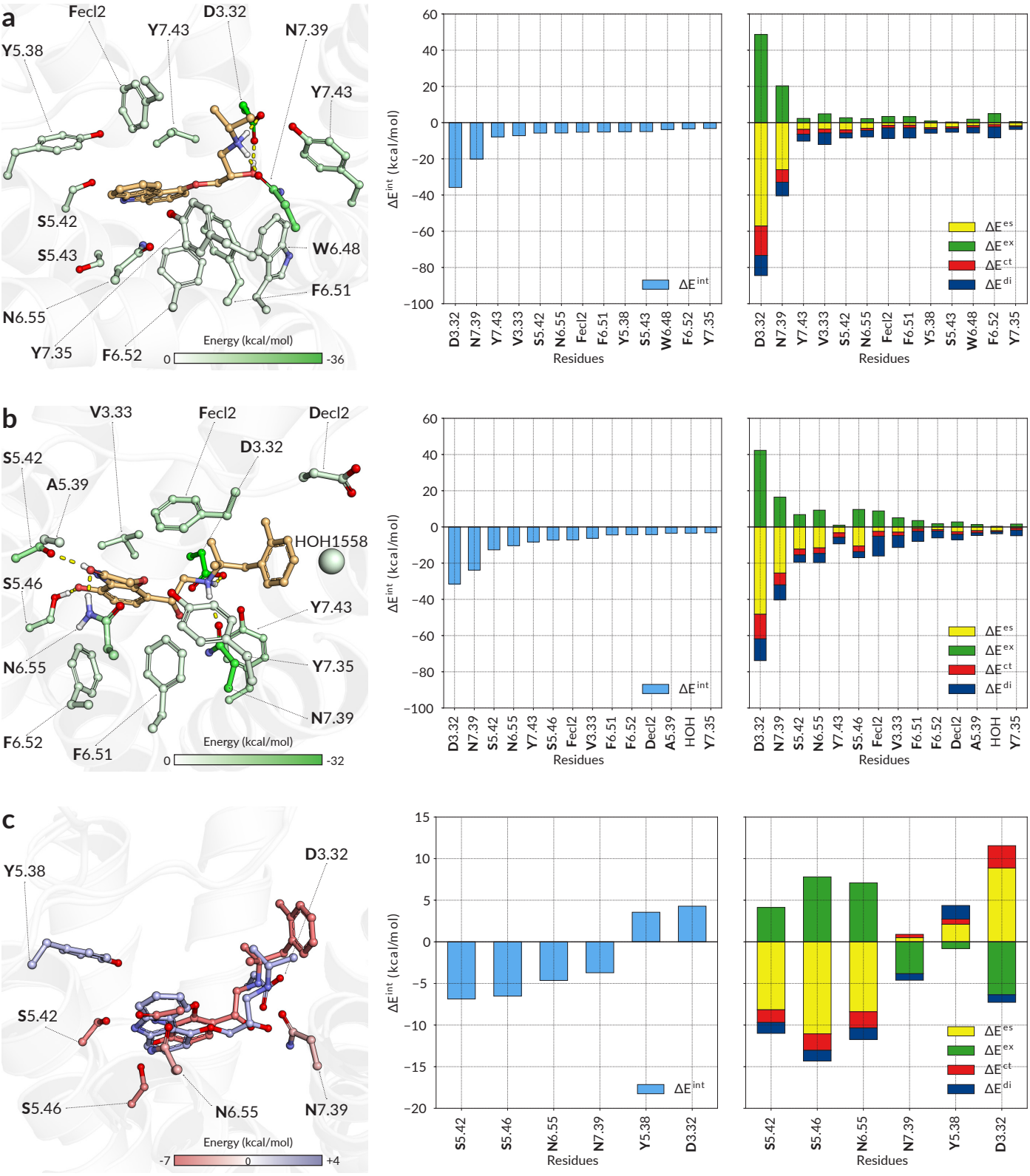


Exchange repulsion (ΔE^{ex})
Repulsive forces between molecules that are close together, mainly due to the overlap of occupied orbitals.



Pair Interaction Energy (PIE)

$$\Delta E_{ij}^{int} = \Delta E_{ij}^{es} + \Delta E_{ij}^{ex} + \Delta E_{ij}^{ct} + \Delta E_{ij}^{di}$$


a
Fecl2
D3.32
Y7.43
N7.39
Y5.38
Y7.43
S5.42
S5.43
W6.48
F6.51
N6.55
Y7.35
F6.52
Energy (kcal/mol)
0
-36
ΔE^int (kcal/mol)
D3.32 N7.39 Y7.43 V3.33 S5.42 N6.55 Fecl2 F6.51 Y5.38 S5.43 W6.48 F6.52 Y7.35
Residues
ΔE^int
ΔE^es
ΔE^ex
ΔE^ct
ΔE^di
b
V3.33
Fecl2
Decl2
D3.32
S5.42
A5.39
HOH1558
S5.46
Y7.43
N6.55
Y7.35
N7.39
F6.52
F6.51
Energy (kcal/mol)
0
-32
ΔE^int (kcal/mol)
D3.32 N7.39 S5.42 N6.55 Y7.43 S5.46 Fecl2 V3.33 F6.51 F6.52 Decl2 A5.39 HOH Y7.35
Residues
ΔE^int
ΔE^es
ΔE^ex
ΔE^ct
ΔE^di
c
D3.32
Y5.38
S5.42
S5.46
N6.55
N7.39
Energy (kcal/mol)
-7
0
+4
ΔE^int (kcal/mol)
S5.42 S5.46 N6.55 N7.39 Y5.38 D3.32
Residues
ΔE^int
ΔE^es
ΔE^ex
ΔE^ct
ΔE^di

FMO DATABASE

2RH1-A

Calculation Name : 2RH1-A-Xray36
Preferred Name : Beta-2 adrenergic receptor
Target Type: SINGLE PROTEIN
Organism: Homo sapiens
Ligand Name:maltose
ligand 3-letter code:MAL
PDB ID: 2RH1
ChEMBL ID: CHEMBL210
UniProt ID: P07550
Base Structure: X-ray
Registration Date: 2019-02-12

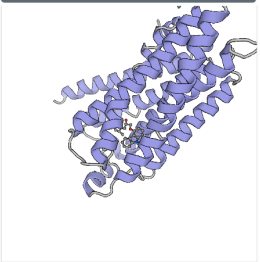
IFIE MAP

Download Files

Modeling method

Optimization	MOE: Amber10:EHT
Restrain	OptHL
Protonation	MOE: Protonate3D
Complement	MOE: StructurePrepareation
Water	No
Automated FMO calculation protocol	Manual calculation

3D Structure



Snapshot

GLU30 DIST:21.144 Total:-12.369 DI_MP2:0.000

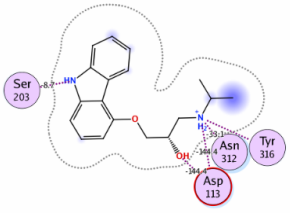
FMO calculation

FMO method	FMO2-MP2/6-31G(d)
Fragmentation	Auto
Number of fragment	441
LigandCharge	CAU=1
Software	MIZUHO/ABINIT-MP 3.0

Total energy (hartree)

FMO2-HF: Electronic energy	-7679721.388843
FMO2-HF: Nuclear repulsion	7501099.115719
FMO2-HF: Total energy	-178622.273124
FMO2-MP2: Total energy	-179138.187531

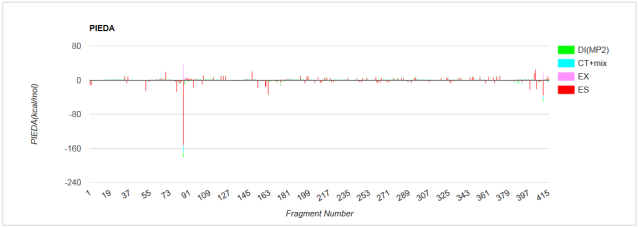
Ligand Interaction



Ligand binding energy by IFIE and PIEDA

IFIE [kcal/mol]	PIEDA [kcal/mol]				Charge transfer value [e]
IFIE SUM	**ES**	**EX**	**CT+mix**	**DI(MP2)**	**q(I=>J)**
-224.935	-196.216	92.389	-45.724	-75.391	0.224

Detail of ligand binding energy by PIEDA

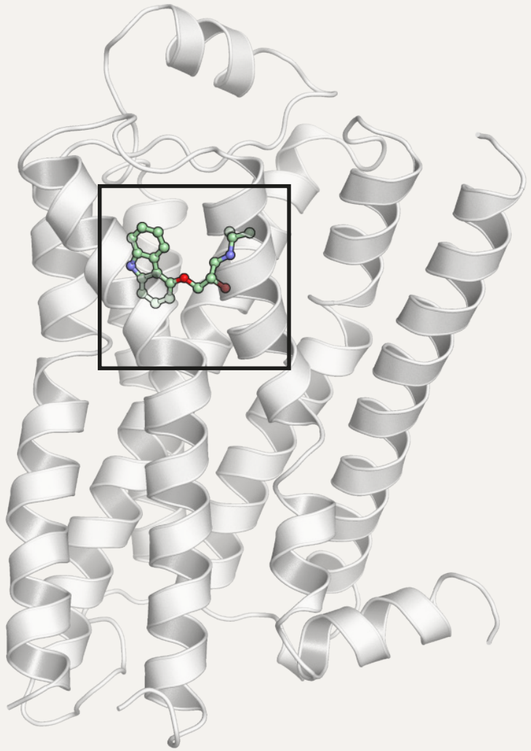


Snapshot

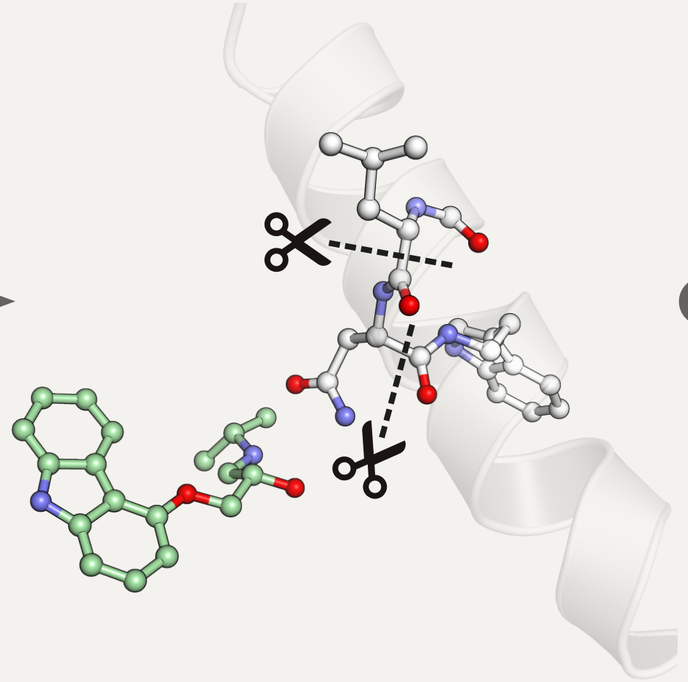
Fragment number of PIEDA/IFIE	Ligand(A:408:CAU)
Distance from Ligand [Å]	Dist <
Interaction energy by IFIE and PIEDA [kcal/mol]	| Total | > | ES | > | EX | > | CT+mix | > | DI(MP2) | >
Fragment charge [e]	FCHARGE q_Mulliken q_NPA q(I=>J)
Residue	Res # RES
Sort	ascending
Graph Options	X Axis Label: Fragment Number; Y Axis Max; Y Axis Min; Display: ES, EX, CT+mix, DI(MP2)

Submit

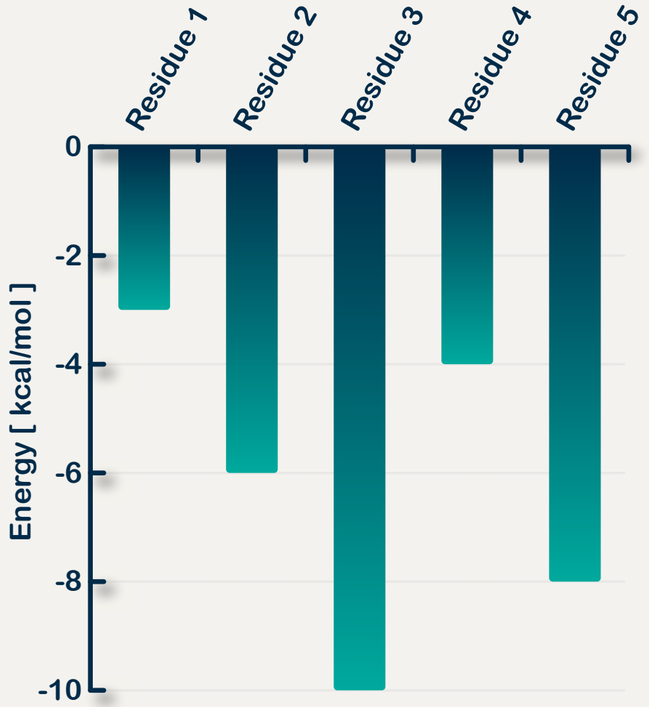
Fragment Molecular Orbital (FMO) Workflow



GPCR-Ligand Complex



Fragmentation



Interaction Energy